Hypothermic liver perfusion

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Abstract: PURPOSE OF REVIEW: The review describes recent developments in hypothermic machine liver perfusion with a special focus on underlying protective mechanisms, and the role of this perfusion technique in high-risk donor-recipient combinations. RECENT FINDINGS: To maximize the number of transplantable donor livers, several centres are exploring new machine preservation techniques. In this context, hypothermic machine perfusion has been recently introduced into the clinical setting of human liver transplantation, and the effect of endischemic cold liver perfusion on posttransplant complications is currently under investigation in two multicentre, randomized controlled trials. In addition, current case series demonstrated promising results regarding the protection from intrahepatic biliary complications, particularly when livers from extended criteria donors including donation after circulatory death grafts were used. Hypothermic machine perfusion may, therefore, help to push the boundaries of acceptance criteria for high-risk donor livers. SUMMARY: In this review, we, first, describe the concept of hypothermic machine liver perfusion and present results from current clinical studies. Next, we provide details of our perfusion approach step-by-step and highlight novel pathways of reperfusion injury and protection. Third, we discuss the impact of this perfusion approach in different clinical scenarios. Finally, we report on recent clinical implementations and future aspects.

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Hypothermic liver perfusion

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Keywords
extended criteria grafts, hypothermic oxygenated perfusion, machine perfusion

INTRODUCTION

Given the shortage of available and suitable organs, transplant physicians are forced to use extended criteria donors (ECD) livers, for example, elderly and steatotic allografts, and organs from donation after circulatory death donors (DCD) [1,2]. Consistently, we observed a shift toward extended and even 'over-extended', or so called high-risk donor livers, frequently offered for transplantation in our aging population [3]. Of note, therefore, the 'standard' liver today would have been similarly classified as 'extended' in the past [4]. Such organs, however, carry an increased risk for primary nonfunction (PNF), early allograft dysfunction (EAD), and intrahepatic biliary complications, with impaired long-term survival rates [1]. An aggressive usage of higher risk grafts remains yet limited, in spite of organ shortage [5]. Based on this, the transplant community is interested to evaluate the impact of new technologies, for example, machine perfusion, on outcome after implantation of high-risk liver grafts. Viability assessment and prediction of graft function are the two challenges to reduce discard rates, and to facilitate well tolerated transplants in the future.

The review focuses, first, on the concept of hypothermic machine perfusion (HMP). Second, we will summarize underlying mechanisms of injury and protection. Third, recent clinical studies are discussed together with the impact of HMP in the context of special clinical situations, when high-risk grafts are used. Finally, we highlight future aspects.

THE CONCEPT OF HYPOTHERMIC MACHINE LIVER PERFUSION

The use of ex-vivo machine perfusion remains investigational in clinical liver transplantation. In general,

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KEY POINTS

- HMP is a valuable tool to improve liver graft quality prior to normothermic reperfusion and transplantation.
- Endischemic HOPE restores cellular energy levels, protects mitochondria from injury during reperfusion, and consequently reduces ROS release and inflammation.
- HOPE treatment prevents development of PNFs and intrahepatic ischemic cholangiopathy in extended human DCD livers.
- Better biomarkers are currently under investigation to predict perfusion duration and later graft function after implantation of HOPE-treated livers.

machine perfusion provides continuous circulation of oxygen, nutrients, and other metabolic substrates during a certain period (Fig. 1) [6]. Prior to 2010, the majority of studies, which reported on machine perfusion, involved only animal models [7–12]. After the first clinical case series of HMP in 2010 [13], however, there has been a rapid development of this technology in clinical settings in Europe and United States [14–17,18▪]. Most clinical investigations target outcomes after ex-vivo machine perfusion at various temperatures using different perfusates and devices [16,19–21]. Despite the overall clinical success of endischemic HMP, optimal temperatures, oxygen concentration, and perfusion protocols are a current topic of debate [19,22,23].

HYPOTHERMIC OXYGENATED PERFUSION TREATMENT: HOW WE DO IT

Our concept of HMP has been introduced into clinical practice in January 2011, when the national DCD transplant program in Switzerland was reestablished [15,24]. This implementation of HMP into the clinic was preceded by over 20 years of basic research [7,12,25–33]. Hypothermic oxygenated perfusion (HOPE) at our centre is, therefore, routinely used to improve the quality of DCD livers, from Maastricht III donors and donation after brain death (DBD) livers with prolonged cold storage and steatotic grafts (Figs. 1 and 2) [16,34▪,35]. Following withdrawal of treatment in theatre, donor cardiac arrest is observed and a 10-min 'hand-off' period is maintained [6]. In next step, brain death is confirmed by our consultant neurologist. Importantly, such scenario leads to a prolonged median total, functional and asystolic donor warm ischemia of 38, 31, and 17 min, respectively, in our DCD cohort (Table 1) [16,36]. No additional heparin is administered to donors before declaration of death. Super rapid cannulation is achieved by laparotomy and insertion of a double balloon catheter into the infrarenal aorta or iliac artery with blocking of the supraceliac aorta. Subsequently, we cut the iliac vein for decompression, and start flushing abdominal organs first with 21 of heparinized saline at room temperature. Afterwards, cold flush is initiated with cooled Institute George Lopez-1 solution (precooled; Fig. 2). During abdominal flush, sternotomy is performed, and the thoracic organs are prepared and procured by the thoracic team. Afterwards, we resect liver, pancreas, and kidneys. Following hepatectomy, and prior to organ packing and transport, an additional liver flush through the hepatic artery, portal vein, and the biliary system is routinely performed (bench procedure in donor centre, Fig. 2). Importantly, the gallbladder is always opened already prior to hepatectomy to flush out potentially toxic bile [37,38]. In the recipient centre, we perform an additional flush through hepatic artery, portal vein, and the biliary system. Following bench

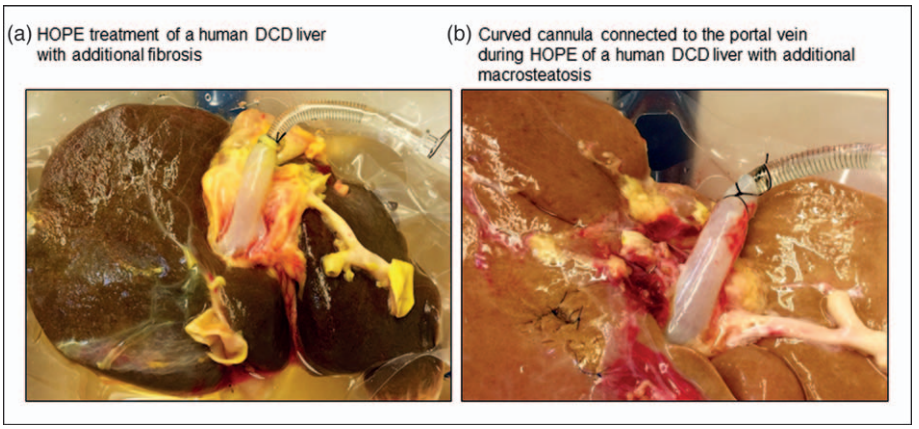

FIGURE 1. HOPE of extended human DCD livers prior to implantation. HOPE perfusion, performed in human DCD liver grafts of different risk profile prior to implantation. (a) 'Normal' DCD liver graft (>30-min fDWIT), (b): Steatotic DCD liver graft. DCD, donation after circulatory death donors; fDWIT, functional donor warm ischemia time; HOPE, hypothermic oxygenated perfusion.

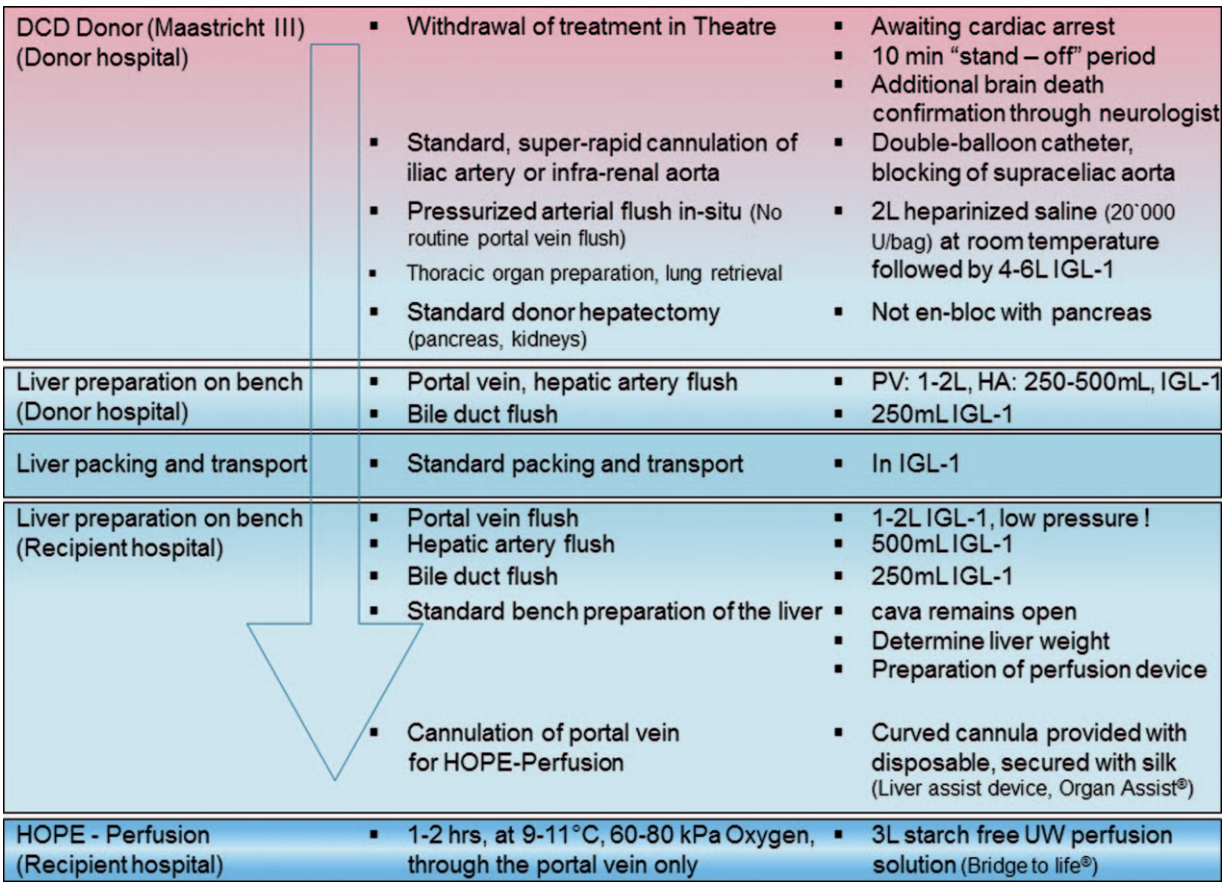

FIGURE 2. HOPE in Maastricht III DCD donor livers – How we do it. Detailed process of DCD liver donation, procurement, bench procedure, and HOPE treatment, as performed at our centre. DCD, donation after circulatory death donors; HA, hepatic artery; PV, portal vein; UW, University of Wisconsin; IGL-1, institute George Lopez-1 solution.

procedure at the transplant centre, we connect the liver to a pressure controlled Liver Assist device (Organ Assist, Groningen The Netherlands), using a curved cannula (Groningen The Netherlands), which is inserted into the portal vein and secured by silk ties (Fig. 2). HOPE is then applied through the portal vein only, using highly oxygenated (60–80 kPa) Belzer machine perfusion solution. The perfusion pressure is limited to maximal 3 mmHg, and permanently controlled by the device to avoid additional endothelial injury [30]. Liver treatment by HOPE is continued during recipient hepatectomy for at least 1 h, but generally until implantation. Prior to HOPE we routinely obtain a liver biopsy to quantify liver graft steatosis and fibrosis. Under special conditions, for example, when DCD liver grafts have additional steatosis, we delay the start of the recipient surgery, to be aware of the biopsy result and observe the liver behaviour during HOPE. In addition to perfusion flow and pressure, further parameters involve perfusate aspartate aminotransferase and alanine aminotransferase and oxygen consumption [15]. For future measurements, we collect perfusate to run absorbance and metabolomics analyses for investigation of new biomarkers, which could help to predict the required perfusion duration. Our group has also analysed the perfusion quality of liver tissue comparing the portal vein only approach with a dual HOPE technique (through hepatic artery and portal vein). As both techniques achieved an equally well and homogenously perfused liver within the first 5 min of HOPE, including the extrahepatic bile duct, we believe currently that perfusion through the hepatic artery is not advantageous in the cold [15,39–42].

PROTECTIVE MECHANISM OF HYPOTHERMIC OXYGENATED PERFUSION

Normothermic reperfusion after ischemia leads to injury in organs, defined as reperfusion injury [43]. Current data suggests an impact of the duration of ischemia before reperfusion on subsequent cell damage following reperfusion. Paradoxically, therefore, although ischemic cells need oxygen to survive, restoration of blood flow causes injury [44].

Table 1. Recent clinical studies on human liver transplantation after graft treatment with hypothermic oxygenated perfusion or hypothermic machine perfusion

Author	Year	Model	Donor warm ischemia (min)	*n*	Temp (°C)	Perfusion solution	Perfusion duration (h)	Perfusion control	Perfusion route	Result
Dutkowski *et al.*	2014	DCD[a]	Total WI: 38 Functional: 31 Asystolic WI: 18	8	10	3 l UW MPS	1–2	Pressure	PV	HOPE-treated extended DCD liver grafts showed significant improved survival and less ischemic cholangiopathy
Guarrera *et al.*	2015	ECD	-	20	4–8	Vasosol	4–7	Flow	PV and HA	HMP significantly decreased EAD, hospital stay and showed significantly less biliary complications
Dutkowski *et al.*	2015	DCD[a]	Total WI: 36 Functional: 31 Asystolic WI: 18	25	10	3 l UW MPS	1–2	Pressure	PV	HOPE-treated extended DCD liver grafts showed comparable good outcomes to matched low-risk primary DBD grafts
De Carlis *et al.*	2017	DCD[b]	OOHCA (15 min), NRP: 135 min	1	10	Celsior	3	Pressure	PV and HA	No biliary complications in the first 5 months. No control group
De Carlis *et al.*	2017	DBD[c]	-	2	10	UW MPS	6 and 8 h	Pressure	PV and HA	Excellent immediate graft function, no complications No control group
Van Rijn *et al.*	2017	DCD[a]	Total WI: 27 Asystolic WI: 15	10	10	4 l UW MPS and 3 mmol/l glutathione	2.1	Pressure	PV and HA	dHOPE treatment restored hepatic ATP, protected from reperfusion injury, and improved 6 and 12-month graft survival
Schlegel *et al.*	2017	DCD[a]	Total WI: 36 Functional: 31 Asystolic WI: 19	50	10	3 l UW MPS	1–2	Pressure	PV	HOPE-treated extended DCD liver grafts showed significant improved 5-year graft survival because of less PNF, HAT, and ischemic cholangiopathy, control group = untreated DCD, matched according to cold storage

Perfusion device: All groups used the liver assist device, apart from J. Guarrera who applies the HMP through a nonpulsataile pump (Medtronic, Minneapolis, Minnesota, USA); Vasosol is based on Belzer's Machine Perfusate (KPS-1, Organ Recovery Systems, Chicago, IL, USA) and is enhanced with α-ketoglutarate, nitroglycerine, L-arginine, N-acetylcysteine, and prostaglandin E1 [17,18▪].
DBD, donation after brain death; DCD, donation after circulatory death donors; dHOPE, dual HOPE (dual = perfusion through HA and PV); EAD, early allograft dysfunction; ECD, extended criteria donors; HA, hepatic artery; HAT, hepatic artery thrombosis; HMP, hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; MPS, machine perfusion solution; OOHCA, out of hospital cardiac arrest (duration), NRP, normothermic regional perfusion in the donor; PNF, primary nonfunction; PV, portal vein; UW, University of Wisconsin; WI, donor warm ischemia.
[a]DCD Maastricht III category.
[b]DCD Maastricht II category.
[c]DBD with prolonged cold storage of 18 h 15 min and 20 h prior to HOPE treatment.

The mechanism behind has been recently discovered, and relates mainly to release of mitochondrial-derived reactive oxygen species (ROS) during the first minutes of normothermic reperfusion (Fig. 3) [29,45–47]. The vast and initiating ROS are probably delivered from mitochondrial complex I, and initiate further injury by hydroxylation of cardiolipin, and opening of the mitochondrial permeability transition pore together with disruption of the energy producing electron transfer [25,30,44,48]. The mechanism of ROS release by complex I was recently reported and was based on an unphysiologic reverse electron flow (RET) between complex II and complex I, caused by accumulation of succinate during ischemia in combination with a high proton motive force in the first minutes of reperfusion [49].

Mitochondrial ROS induce downstream inflammation process, leading to release of danger-associated molecular patterns and consecutively activation of toll-like receptors on nonparenchymal liver cells [34▪]. Subsequently, an innate immune response contributes to activation of lymphocytes and formation of scar tissue by activation of stellate cells [50].

The protective effect of HOPE appears several folded: first, during HOPE, we observed very low levels of RET despite high levels of oxygen, probably related to hypothermia-induced low proton-motive forces. Second, HOPE leads to metabolism of accumulated succinate and to significant upload of ADP and ATP levels within 1–2 h (Fig. 3) [17,31,34▪].

Third, after HOPE treatment, electron donors are decreased and high nucleotide levels restored which leads to a reduced RET and less ROS release during reperfusion [30,31,33,34▪,51]. Numerous downstream inflammation processes appear, therefore, decreased after transplantation of HOPE treated liver grafts, including the immune response, resulting in less graft rejections in animal models and also in human DCD liver transplants [32,36].

CLINICAL DATA

Several promising nonrandomized clinical studies on HMP in liver transplantation have been published over the past 3 years [14,16,17]. Although some variability exists in the perfusion technique, all clinical HMP series to date have reported improved outcomes with reduction in EAD, biliary complications, reduced hospital length of stay, and improved graft survival [14,15,17,34▪]. Following the very first clinical application of hypothermic dual perfusion (portal vein and hepatic artery) in the 20 standard DBD human livers, the group of James Guarerra reported recently that such perfusion approach appeared to be also protective in marginal DBD (ECD) liver grafts [14].

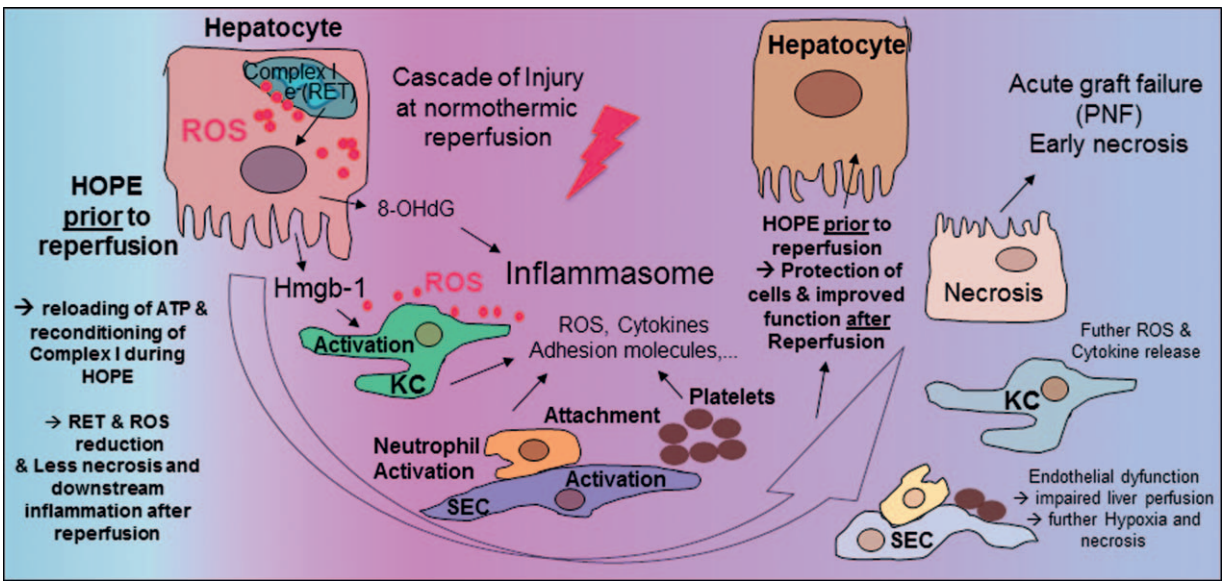

FIGURE 3. Mechanism of injury following ischemia/reperfusion and protection through hypothermic machine perfusion approaches. HOPE treatment provides a reconditioning of mitochondria prior to normothermic reperfusion. At the end of HOPE, ADP and ATP have been recharged together with an increased metabolism of succinate. HOPE-treated livers are, therefore, protected from ROS release, downstream mediator activation, and inflammation. HOPE-treated livers experience consecutively significantly less PNF, less necrosis, and less chronic inflammation. Hmgb-1, high-mobility group box 1 protein; HOPE, hypothermic oxygenated perfusion; KC, kupffer cell; PNF, primary nonfunction; RET, reverse electron flow; ROS, reactive oxygen species; SEC, Sinusoidal endothelial cell; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

Importantly, cold machine perfusion was applied after cold storage and performed without active oxygenation, but the perfusate contained sufficient oxygen because of an open system with contact to room air. Recipients in the perfusion group experienced less biliary complication after implantation of marginal DBD livers [14]. Consistent with these results, HOPE treatment has been shown by our group to be protective in extended human DCD liver grafts, despite long donor warm ischemia times, with no occurrence of intrahepatic biliary complications when compared with matched unperfused DCD livers [16].

Such results were recently paralleled by the report from Groningen, where authors also showed protection of human DCD livers in the Netherlands (Table 1) [17]. In total, 10 human DCD livers were perfused by dual HOPE and liver recipients were protected from graft loss because of intrahepatic biliary complications in contrast to 20 nonperfused controls [17]. In addition, our group recently reported the first 5-year outcome study with more than 50 human DCD livers transplanted after HOPE treatment [36]. Compared with matched, unperfused controls, HOPE livers developed significantly less PNF, hepatic artery thrombosis, and importantly intrahepatic biliary complications (Table 1) [36]. Although these reports lack randomization, two randomized controlled trials are currently on their way (hope-liver.com – Zurich, Groningen Institute for Organ transplantation). In addition, the impact of HOPE is explored in clinical and experimental models of heart, lung, and kidney transplantation [52–55].

The development of innovative portable machine perfusion devices has opened the door for a wider use of machine perfusion in other countries. In this context, a HOPE approach was recently tested in Maastricht Type II DCD livers following normothermic regional perfusion, and after extended cold storage in Italy [18▪,56].

WHICH LIVERS REQUIRE MACHINE PERFUSION?

Liver injury already starts during brain death, and increases with every day a potential organ donor remains in the ICU [1]. Additional injury accumulates throughout the entire process of organ procurement, transport, bench preparation, and implantation [1,6,37]. Although routine cold storage has served as gold standard in organ preservation in the past, physicians become more and more aware of the risk of cold storage for extended criteria grafts, for example, steatotic, aged, or DCD livers [1]. The disadvantage of static preservation is related to the lack of cellular energy and nutrients, leading to continuous nucleotide breakdown, lactate acidosis, calcium accumulation, calpain activation, and sinusoidal endothelial injury [51]. The maximum time period, where cold storage can be safely applied is therefore limited [7], but standard grafts cope relatively well with 8–10 h of cold static preservation.

In contrast, livers with additional previous ischemia in donors, or livers that produce high levels of acetyl CoA, for example, steatotic grafts, trigger an overload of electron donors, and consecutively suffer from high levels of mitochondrial ROS during reperfusion [47]. Such livers particularly benefit from ex-vivo graft treatment to avoid significant reperfusion injury [57,58]. Of note, many transplant programs experience nowadays a shift toward more marginal or ECD grafts, offered for transplantation (Fig. 4) [4,59,60]. Machine perfusion could, therefore, contribute to optimize high-risk donor and recipient combinations. However, reliable clinical thresholds are not available yet, and multiple definitions for extended or high-risk liver grafts exist [3]. Of note, DCD livers with limited donor warm ischemia and relatively low age can achieve excellent outcomes without any additional perfusion technique ($\leq$60 years donor age, $\leq$ 20-min functional donor warm ischemia, $\leq$ 6 h cold ischemia, $\leq$5% macrosteatosis).

In contrast, we would also classify DBD livers older than 80 years, or DBD livers with more than 20% of macrosteatosis to the group of 'ECD livers', and suggest to perform ex-vivo machine perfusion preservation in those livers (Fig. 4).

In the future, machine perfusion techniques will, therefore, enable physicians to accept more risky allografts (overextended DCD). To promote a universal application of HMP, the benefits should be assessed objectively and compared to other perfusion approaches.

CONCLUSION AND FUTURE ASPECTS

Machine perfusion receives currently high interest in the transplant community, as many organs carry additional injury. Convincing machine perfusion technologies should offer true repair of damaged organs, should allow to measure metabolic function before implantation, and should potentially prolong well tolerated preservation for up to 12 h to address logistic hurdles. At the same time, however, perfusion technology needs to be as simple and affordable as possible with utmost benefit compared to cold storage. Current and future perfusion technologies should be evaluated and compared based on these criteria. An optimized perfusion approach is likely to be widely accepted by many transplant

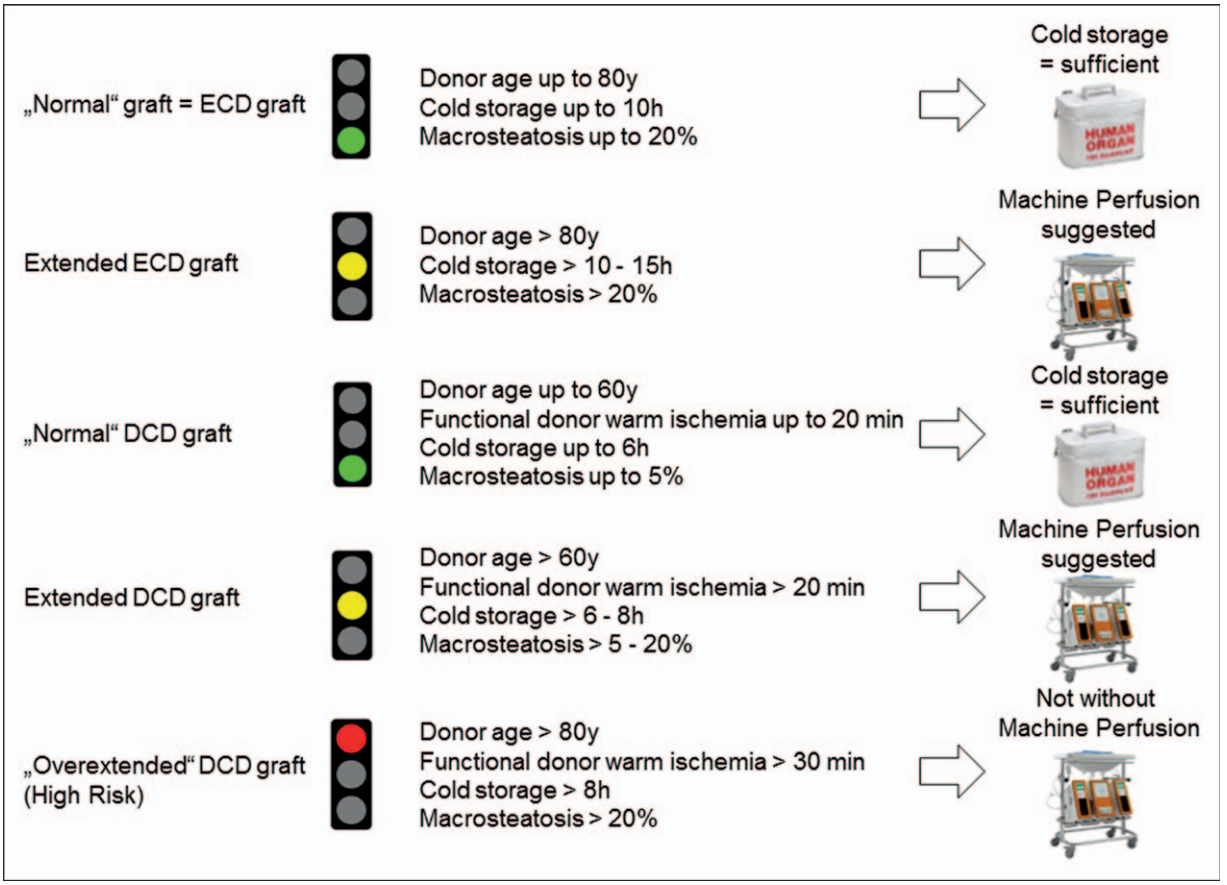

FIGURE 4. Different risk categories of donor liver grafts and the suggested preservation strategy. Normal DBD and DCD liver grafts are routinely well preserved with cold storage and do not necessarily require machine perfusion, unless a very sick, high MELD recipient has been allocated. Livers with increasing risk at all ends, including advanced donor age in combination with prolonged warm and cold ischemia and additional steatosis benefit graft treatment prior to normothermic reperfusion. Particularly, DCD and ECD grafts, where the cumulative risk appears too high should receive machine perfusion treatment and functional assessment prior to implantation. DCD, donation after circulatory death donors; ECD, extended criteria donors.

programs with high consequences on organ supply and outcome.

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Conflicts of interest

There are no conflicts of interest.

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- ■■ of outstanding interest

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